wherein the hematopoietic cells are freshly isolated lymphocytes, stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof.

31. (Amended) A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, and iii) hematopoietic cells selected from the group consisting of freshly isolated lymphocytes, stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof.

Please add the following new claims:

37. (New) The cryopreservation medium of claim 1 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.

38. (New) The composition of ~~claim 14, 15 or 31~~ wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.

Remarks

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks herein, is respectfully requested. Claims 1, 14-15 and 31 are amended, claims 13 and 25 are canceled, and claims 37-38 are added. The pending claims are claims 1-12, 14-24 and 26-38.

Amended claims 1, 14-15 and 31 are supported by originally filed claims 1, 14-15 and 31, respectively, and claims 13 and 25.

New claims 37-38 are supported by originally-filed claims 13 and 25.

Applicant has reviewed the Notice of Draftsperson's Patent Drawing Review (PTO-948). Drawings which comply with 37 C.F.R. § 1.84(g) and § 1.84(p) will be filed in a timely manner after receipt of a notice of allowable subject matter.

The Examiner rejected claims 1-2, 4-16 and 18-34 under 35 U.S.C. § 112, first paragraph. In particular, the Examiner asserts that while the specification enables arabinogalactan

derivatives, it does not reasonably provide enablement for biological and functional equivalents of arabinogalactan, which include compounds structurally unrelated to arabinogalactan. This rejection is respectfully traversed.

As evidence that Applicant's disclosure would enable the art worker to identify biological or functional equivalents of arabinogalactan falling within the scope of the claims, the Examiner is requested to consider Applicant's specification. The specification discloses that "arabinogalactan, a biological or functional equivalent thereof" includes naturally occurring or synthetic arabinogalactan (AG), portions of AG, and chemically or biochemically modified AG or portions thereof (page 3). It is also disclosed that agents useful in the compositions and methods of the invention are cryoprotective agents for hematopoietic cells which are present in an amount that is effective to promote a high survival rate for the cryopreserved cells (pages 2-3). Exemplary cryopreservation media are described at pages 14-16 and in Example 1 of the specification, pre-freezing, freezing and thawing methods are disclosed at pages 21-24 of the specification, and methods to determine the viability and recovery of cells after freezing and thawing are discussed at pages 24-25 and in Examples 1 and 2 of the specification. Thus, one of ordinary skill in the art in possession of Applicant's specification would be apprised of how to identify biological or functional equivalents of AG. Therefore, Applicant's specification is enabling.

It is respectfully submitted that the pending claims are in conformance with 35 U.S.C. § 112, first paragraph. Thus, withdrawal of the rejection of the claims under § 112(1) is respectfully requested.

The Examiner rejected claims 1-34 under 35 U.S.C. § 102(b) as anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as obvious over, the LAREX Material Safety Data sheet or WO 97/35472. These rejections, as they may be maintained with respect to the pending claims, are respectfully traversed.

The LAREX Material Safety Data sheet (which, as submitted on Form 1449 on April 26, 2000, included 3 pages, 2 of which were the Material Safety Data Sheet and one of which was a Technical Data Sheet) relates that Cellsep™ powder contains at least 99% AG, and that AG is approved as a food additive by the FDA. The Technical Data Sheet discloses that Cellsep™ powder is a medium for density gradient cell separation which provides superior resolution of a

wide variety of cell types and cellular organelles. Density gradient media are employed to separate organelles and/or cells such as red and white blood cells based on their buoyant density in the solution used for separation. The Technical Data Sheet also discloses that Cellsep™ isotonic solutions are available for lymphocytes and platelets. No mention is made in either the Material Safety Data Sheet or the Technical Data Sheet of cryopreservation solutions or methods to cryopreserve cells.

WO 97/35472 relates to the use of AG in cryopreservation media for immortalized somatic cells. Although WO 97/35472 indicates that the described media may be employed with a variety of cell types including human cells (page 5, line 2) and blood cells (page 10, line 4), the only data provided in the WO 97/35478 specification is for seven lines of immortalized mammalian cells (page 13). These included three lines derived from rodent epithelial cells, a line derived from mink fibroblasts, a line derived from human fibroblasts, a line derived from bovine endothelial cells (CPAE cells), and a line derived from murine pre-neoplastic mammary cells.

These seven lines were frozen in 6 different media (Table 1). For media containing AG, it is disclosed that AG was prepared as a 50% w/v concentrated stock dissolved in a buffered isotonic salt solution. This stock was used directly (medium 3, i.e., 50% AG) or in combination with other components. Medium 4 has 20% AG and 10% DMSO; medium 6 has 15% AG and 20% serum, medium 2 has 10% AG and 20% DMSO; and medium 5 has 10% AG, 10% DMSO and 20% serum. Medium 1 has 10% DMSO and 20% serum (no AG).

With respect to immediate post-thaw viability for all cell types tested, it is disclosed that there was no difference in post-thaw viability for 4 of the media relative to "the industry standard" (cell culture medium + serum + DMSO) (page 14), however, cells frozen in media with AG and serum had reduced viability. It is also noted that there was "substantially no difference" in plating efficiency at day 1 for 6/7 of the cell types (page 14). At six days post-thaw, it is disclosed that there was "substantially no difference" between treatment groups (page 15). Table 2 shows the ranking of the media with respect to growth rates (Day 6/Day 1) for CPAE cells (media 3 > media 5 > media 2 > media 1 > media 4 > media 6). WO 97/35472 concludes that AG "can be used to replace serum in a standard freezing medium, in a formulation

with DMSO, for all cell types studied" (emphasis added; page 15) and that freezing in 50% w/v AG was better or equivalent to the standard media for 5/7 cell types tested (page 15).

Compositions and methods to cryopreserve one cell type are not necessarily the same as the methods and compositions employed for other cell types, as each cell type has different physical properties. For example, at page 97 of Sputtek et al. (In: Clinical Applications in Cryobiology, CRC Press, 1991), it is noted that the conditions employed to freeze red blood cells do not result in viable white blood cells. Further, in Hubel (Transfusion Med. Rev., 11, 224 (1997)), it is disclosed that the permeability of the membrane to water and penetrating cryoprotective agents influences the osmotic response of the cells during the introduction and removal of the cryoprotective solution (page 226). It is also disclosed that during the freezing process, the permeability of the membrane is important in determining the water content of the cell, which in turn influences intracellular ice formation or the extent of dehydration of the cell (page 226). Hubel also discloses that the membrane permeability parameters for a number of blood cell types including lymphocytes was found to be distinctive (see Table 1). Moreover, Figure 3 in Hubel provides data showing that freshly isolated $CD34^+$ cells and cultured transduced $CD34^+$ cells have different physical characteristics at different temperatures, including water permeability, cell volume and the osmotically inactive cell volume fraction (page 228).

Figure 1 in Applicant's specification depicts peripheral blood lymphocyte recovery as a function of AG concentration (5-25%). Notably, 15% w/v AG and not a higher concentration of AG, led to the highest cell recovery, which contrasts with WO 97/35472 in which 50% w/v AG was better or equivalent for 5/7 immortalized non-hematopoietic mammalian cells. Differences in cell recovery for activated peripheral blood lymphocytes versus cultured peripheral blood lymphocytes and genetically altered peripheral blood lymphocytes versus normal peripheral blood lymphocytes in the same AG-containing cryopreservation medium and relative to DMSO-containing medium is shown in Tables 3 and 4, respectively, of Applicant's specification. Further, the freezing rate for DMSO-containing solutions leading to high cell recoveries is different than for AG-containing solutions (Tables 2 and 3 of Applicant's specification).

As the cryopreservation properties of cells other than hematopoietic cells and of hematopoietic cells in any particular cryopreservation media are likely different, the disclosure of

AG-containing "density gradient separation media", AG-containing "isotonic solutions" for lymphocytes or certain AG media useful for cryopreservation of immortalized non-hematopoietic mammalian cells does not enable a cryopreservation medium for hematopoietic cells, e.g., freshly isolated peripheral blood lymphocytes, or a method for cryopreserving hematopoietic cells. Hence, neither the LAREX Material Safety and Technical Data Sheets or WO 97/35472 anticipate or render obvious Applicant's invention.

Therefore, the Examiner is respectfully requested to withdraw the § 102(b) and § 103(a) rejections of the claims.

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6959) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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CERTIFICATE UNDER 37 C.F.R. 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 26th day of February, 2001.

Dawn M. Poole
Name

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Signature